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DIAGNOSIS AND PREVENTION OF INFECTION BY PHLEBOTOMUS FEVER
GROUP VIRUSES

ANNUAL REPORT

DAVID H.L. BISHOP

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<p>The sequence of the M RNA of the M12 derivative of ZZ548 has been determined and compared to that of ZZ501. The data indicate that the RNA and its gene product are comparable for both viruses. Comparison with the sequence of the parent ZZ548 virus is in hand.</p> <p>Expression of the PT N and NS_g proteins has been achieved using baculovirus expression vectors based on AcNPV. High Level expression of both proteins has now been achieved. Expression of the PT M gene products, G1 and G2, in vaccinia vectors has also been achieved.</p>				
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I. SUMMARY

The objectives of the contract are to develop new diagnostic procedures and new vaccine strategies pertinent to selected Phlebotomus fever (PHL) group viruses, viruses that constitute the *Phlebovirus* genus of the negative-sense RNA virus family Bunyaviridae [Bishop *et al.*, 1980]. This genus of viruses includes members that are known animal [including human] pathogens and that are therefore of consequence to military and civilian personnel in particular regions of the world [Bishop & Shope, 1979].

The development of new diagnostic tools for the specific identification of PHL group viruses is being investigated using eukaryotic expression vectors and cDNA clones of particular phlebovirus genes that are available to us. Two expression systems are under study, vaccinia and the insect specific baculovirus expression vectors. So far, it has proven difficult to express the complete M RNA gene products of Punta Toro (PT) phlebovirus using either vector. Recombinant vaccinia viruses have, however, been made that express part of the PT M RNA, producing the PT glycoproteins G1 and G2. The expressed products have been characterized. These studies were undertaken in conjunction with staff of Professor R.W. Compans of the University of Alabama in Birmingham. The expression of PT S RNA gene products supported by this contract and its immediate predecessor [DAMD-17-G-4035, see Overton *et al.*, 1987] using *Autographa californica* nuclear polyhedrosis virus [AcNPV] baculovirus vectors is also reported.

The objective of developing new vaccine strategies for PHL group viruses is being realized through the characterization of an attenuated, candidate vaccine derivative of Rift valley fever (RVF) phlebovirus [the M12 mutant of RVFV isolate ZZ548]. This candidate vaccine was previously developed by staff of the Principal Investigator working at USAMRIID [Caplen *et al.*, 1984]. In this reporting period the complete sequence of the viral M RNA of the M12 RVFV derivative has been obtained.

II. REPORT

A. Introduction

Our analyses have been directed towards developing new strategies for phlebovirus vaccine development. Initial studies, supported by prior U.S. Army Medical Research and Development contracts, were aimed at characterising the

genome and structural components of phleboviruses. With the demonstration of a tripartite RNA genome for phleboviruses, genetic analyses were then undertaken to delineate the coding strategies of the viral RNA species and to determine if recombinant viruses could be obtained and used for vaccine purposes. By analyses of intertypic reassortant PT viruses we initially showed that the viral 7×10^5 dalton small [S] RNA species codes for the viral 26.9×10^3 dalton nucleocapsid [N] protein. The results of cloning and sequencing the S RNA of PT phlebovirus [Ihara *et al.*, 1984] confirmed these data and showed that an open reading frame in the *viral-complementary* S RNA sequence coded for N [S mRNA]. A *second open reading frame* in the *viral-sense* strand coding for a 29.1×10^3 dalton non-structural protein, NS_S [Ihara *et al.*, 1984] was also identified. The two subgenomic PT S induced mRNA species [one viral-complementary, the other viral-sense] were characterized with regard to their 3' and 5' end sequences and rates of synthesis in infected cells [Ihara *et al.*, 1985a; Emery & Bishop, 1987].

The results of cloning and sequencing the 2×10^6 dalton middle [M] size RNA of PT virus [Ihara *et al.*, 1985b] demonstrated that the PT M RNA codes for the 50.70×10^3 dalton viral glycoproteins, G1 and G2. A 30×10^3 dalton non-structural protein, NS_M, that constitutes the amino terminal end of the PT glycoprotein precursor was also identified. The data showed that the order of the PT M gene products was NS_M-G1-G2. The PT M viral-complementary mRNA species were characterized [Ihara *et al.*, 1985a]. The results obtained for the PT M RNA correlated with the data reported by Collett and associates [1985] for RVFV M RNA and its gene products [RVFV M gene order: NS_M-G2-G1]. Although not proven, it is assumed that the phlebovirus 3×10^6 dalton large [L] RNA species codes for the 200×10^3 dalton large protein [a putative transcriptase component] found in viruses.

The genetic studies, including interference assays, reported from the work conducted in the previous contract, indicated that although intertypic reassortant viruses could be obtained, heterotypic virus interactions were not demonstrable (i.e., heterotypic phleboviruses did not interfere with each other and did not reassort their genomes in dual virus infections). Although not all phleboviruses were tested for genetic interactions, the results did not hold out much hope for this approach for vaccine development. Consequently, an alternative approach, that of developing candidate, including subunit, vaccines has been proposed for this contract. These objectives, together with research directed towards the

development of new and specific diagnostic reagents form the principal objectives of this contract.

B. Results from this Reporting Period.

The sequence of the M RNA of the M12 candidate vaccine of RVFV

Based on analyses of cDNA clones, the sequence of the M RNA of the ZZ501 strain of RVFV has been reported by Collett and associates (1985). In order to characterize the candidate RVFV vaccine that was produced from the related ZZ548 RVFV [Caplen *et al.*, 1984], we have sequenced the M RNA of the M12 derivative. The sequence was obtained by analyses of λ cDNA clones. The data are shown in Fig. 1 [manuscript in preparation]. Based on the cDNA sequence analyses the RVFV M12 M RNA is deduced to be 3885 nucleotides long (mol. wt. 1.38×10^6 , base composition: 27.3% A, 27.2% U, 25.4% G, 20.1% C) and has 3'- and 5'-terminal sequences that are complementary for some 9 residues. The viral RNA codes in its viral-complementary sequence for a single long gene product [the viral glycoprotein precursor] that is comprised of 1197 amino acids (130,000 daltons). The gene product is abundant in cysteine residues but has few potential asparagine-linked glycosylation sites. It is initiated by a methionine codon at nucleotide residues 21-23. These data are similar to those reported for RVFV ZZ501 M RNA [as ammended by recent studies at USAMRIID, J. Dalrymple, personal communication]. However, unlike the ZZ501 sequence, there is a second AUG codon [residues 9-11] in the viral-complementary sequence *upstream* of the M12 M gene product [Fig. 1]. It is followed by valine and histidine codons then a translation terminator [TAA, residues 18-20]. Even discounting this region the 5' non-coding region of the RVFV M viral-complementary RNA is short (20 nucleotides); the 3'-noncoding sequence is much longer (271 nucleotides). No other large open reading frame has been identified in either the viral, or viral-complementary RVFV M RNA sequences.

Limited amino-terminal sequence analyses of the ZZ501 RVFV glycoproteins indicated the gene order and potential cleavage sites in the glycoprotein precursor. The data suggested the existence of a 23×10^3 dalton polypeptide (designated NS_M) in the glycoprotein precursor that precedes the RVFV G2 and G1 proteins (i.e., gene product order: NS_M-G2-G1). Examination of the sequence of the M12 gene product indicates that the ZZ548 M12 and ZZ501 sequences are closely related and that the amino terminal sequences of the viral

Fig. 1. Sequence of the M cDNA of RVFV M12

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M V H * M Y V L L T I L I S V L V C E A I I R V S L S S T R E E T C F G D S
ACACAAGATGGTGCATTAAATGTATGTTTTATTAACAATCTAACCTGGTTCCTGGTGTGTGAAGCGATTATTAGAGTGTCTCTAAGCTCCACAAGAGAGAGACCTGCTTTGGTGACT
10      20      30      40      50      60      70      80      90      100     110     120

T N P E M I E G A W D S L R E E E M P E E L S C S I S G I R E V K T S S D E L Y
CCACTAACCCAGAGATGATTGAAGGAGCTTGGGATTCACTCAGAGAGGAGGAGATGCCGGAAGGAGCTTCCTGTTCTATATCAGGCATAAGAGAGTTAAGACCTCAAGCCAGGAGTTAT
130     140     150     160     170     180     190     200     210     220     230     240

R A L K A I I A A D G L N N I T C H G K D P E D K I S L I K G P P H K K R V G I
ACAGGGCATTAAAGCCATCTTGTCTGATGGCTTGAACAACATCACCCTGCCATGCTAAGGATCCTGAGGACAAAGATTCCCTCATAAAGGGTCTCTCCACAAAAGCGGGTGGGGA
250     260     270     280     290     300     310     320     330     340     350     360

V R C E R R R D A K D I G R K T M A G I A M T V L P A L A V F A L A P V V F A E
TAGTTCGGTGTGAGAGACGAAGAGATGCTAAGCAATAGGGAGAAACCATGGCAGGGATTGCAATGACAGTCTTCCAGCCTTAGCAGTCTTTGCTTTGGCACCTGTGTCTTTGCTG
370     380     390     400     410     420     430     440     450     460     470     480

D P H L R N R P G K G H N Y I D G M T Q E D A T C K P V T Y A G A C S S F D V L
AAGACCCCATCTCAGAAACAGACAGGAGGGGACACACTACATTGACGGGATGACTCAGGAGGATGCCACATGAAACCTGTGACATATGCTGGGGCATGTAGCAGTTTGTATGCT
490     500     510     520     530     540     550     560     570     580     590     600

L E K G K F P L F D S Y A H H R T L L E A V H D T I I A K A D P P S C D L L S A
TGCTTGAAGAAAGGAAATTTCCCTTTTCCAGTGTATGCTCATAGAACTCTACTAGAGGAGTTCAGACACCATCATTCGAAGGCTGATCCACCTAGCTGTGACCTTCTGAGTG
610     620     630     640     650     660     670     680     690     700     710     720

H G N P C H K E K L V M K T H C P N D Y Q S A H H L N N D G K M A S V K C P P K
CTCATGGGAACCCCTGCATGAAGAGAACTCGTGTATGAAGACACACTGTCCAAATGACTACCACTCAGCTCAGCTCATCACCTCAACCAATGACGGGAAPATGCTTCAGTCAAGTCCCTCTCTA
730     740     750     760     770     780     790     800     810     820     830     840

Y G L T E D C N F C R Q M Y G A S L K K G S Y P L D D L F C G S S E D D G S K L
AGTATGGGCTCAGTGAAGACTGCAACTTTTGTAGGCAGATGACAGGTGCTAGCCTGAAGAGGGGCTTTATCCTCTCCAGACTTGTTTTGTCAAGTCAAGTGAGGATGATGATCAAAAT
850     860     870     880     890     900     910     920     930     940     950     960

K T K M K G V C E V G V Q A L K K C D G D L S T A H E V V P F A V F K N S K K V
TAAATCAAAATGAAGGGGCTCTGCAAGTGGGGGTTCAAGCACTCAAAAAGTGTATGGCCAACTCAGCACTGCACATGAGGTTGTGCTCTTTGAGTGTTTAAGAACTCAAGAAAGG
970     980     990     1000    1010    1020    1030    1040    1050    1060    1070    1080

V L D K L D L K T E E N L L P D S F V C F E H K G Q Y K G T H D S G Q I K R E L
TTTATCTTGATGAAGCTTGACCTTAAGACTGAGGAGAACTGTCTACCAAGACTCATTGTCTGTTTCGAGCATAAGGGACAGTACAAGGAACATGGAGTCTGTGTAGACTAAGAGGAGG
1090    1100    1110    1120    1130    1140    1150    1160    1170    1180    1190    1200

K S F D I S D C P K I G G H G S K K C T G D A A F C S A Y E C T A Q Y A N A Y C
TCAGAAAGCTTTGATATCTCTCAGTGCCTCAAGATTTGAGGACATGGTAGTAGAAGTGCACCTGGGACGACGACATTTTGTCTGCTTATGAGTGCACCTGCTCAGTACGCCAATGCTTAT
1210    1220    1230    1240    1250    1260    1270    1280    1290    1300    1310    1320

S H A N G S G I V O I O V S G V W K K P L C V G Y E R V V V K R E L S A K P I O
GTTTACATGCTTATGGGTGAGGATTTGTGAGATACAAATATCAGGCTCTGGAAAGAGGCTTTATGTGTAGGGTTTAAAGAGTGGTGTGTGAAGAGAACTCTCTGCAAGCCCATCC
1330    1340    1350    1360    1370    1380    1390    1400    1410    1420    1430    1440

R V E P C T T C I T K C E P H G L V V R S T G F K I S S A V A C A S G V C V T G
AGAGAGTGAAGCTTGCACAACTTGTATAACCAATGTGAGCCTCATGGATTGGTTGTCCGATCAACAGGGTTCAAGATATCATCAGCAGTTGCTTGTGCTAGCGGAGTTTGGTCAAG
1450    1460    1470    1480    1490    1500    1510    1520    1530    1540    1550    1560

S Q S P S T E I T L K Y P G I S D S S G G D I G V H M A H D D Q S V S S K I V A
GATCGCAGAGTCTTCCACCGAGATTACACTCAAGTATCCAGGATATCCAGCTCTCTGCGGGGACATAGGGGTTACATGGCACACGATGATCAGTCACTTAGCTCCAAATAGTAG
1570    1580    1590    1600    1610    1620    1630    1640    1650    1660    1670    1680

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Fig. 1 (Contd)

H C P P Q D P C L V H D C I V C A H G L I N Y D C H T A L S A F V V V F V F S S
 CTCACGTGCCCTCCCCAGGACCCGCTTAGTGGATGACTGGCATAGTGTGTGGCTGATGATAAATACCAAGTGCACACTGCTTCAGTGGCTTTGTGTGTGTATTCAGTT
 1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800

I A I I C L A I L Y R V L K C L K I A P R K V L N P L M W I T A F I R W I Y K K
 CTATTGCAATAATTGTTTAGCTATTCTTTATAGGGTGGTTAAGTGGCTGAAGATTTGCCCAAGGAAGTTCTGAATCCACTAATGTGGATCAGAGCTTTCATCAGATGGATATAGA
 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920

M V A R V A D N I N D V N K E I G W H E G G Q L V L G N P A P I P R H A P I P R
 AGATGGTTGCCAGAGTGGCAGACAACATTAATCAAGTGAACAGGGAATAGGATGGATGGGAAGGTCAGTTGGTTCTAGGGAACCTGGCCCTATTCTCTGCTGAGCCCAATCCAC
 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040

Y S T Y L M L L L I V S Y A S A C S E L I Q A S S R I T T C S T E G V N T K C R
 GTTATAGCAGATACCTGATGTATTATTGATTGTCTCATATGCAATCAGCATGTTTCAGAACTGATTCAGGCAAGCTCCAGAAATCACCAGTTGCTCTACAGAGGGTGTAAACACCAAGTGA
 2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160

L S G T A L I R A G S V G A E A C L M L K G V K E D Q T K F L K L K I V S S E L
 GACTGTCTGGCAGACGATTGATCAGAGCAGGCTCAGTTGGGGCAGAGGCTTGTGTATGTGAAGGGGTCAGGAAGATCAACCAAGTCTTAAAGTTAAACCTTCAAGTGAAGC
 2170 2180 2190 2200 2210 2220 2230 2240 2250 2260 2270 2280

S C R E G Q S Y W T G S F S P K C L S S R R C H L V G E C H V N R C L S W K D N
 TATCATGCAAGGAGGAGGAGGATTTAGGACTGGTCTTTAGCCCTAAATGTTTGAAGCTCAAGGAGATGCCACCTTGTGGGGAAATGCCATGTGAATAGGTGTCTGTCTTGSAGSSACA
 2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400

E T S A E F S F V G E S T T H R E N K C F E Q C G G W G C G C F N V N P S C L F
 ATGAACCTCAGCAGAGTTTTCATTTGTTGGGGAAGGACGACCATGGAGAGAATAGTGTTCAGCAATGTGGAGGATGGGGTGTGGTGTTCATATGTGAACCATCTTGTCTAT
 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 2510 2520

V H T Y L Q S V R K E A L R V F N C I D W V H K L T L E I T D F D G S V S T I D
 TTGTGCACACCTATCTGCAGTCAGTTAGAAAAGAGGCCCCTAGAGTTTTCAGCTGATCGCACTGGGTGCATAAAGCTCACTCTAGAGATCAGAGACTTGTATGGCTCTGTTTCAACATGA
 2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640

L G A C S S R F T N W G S V S L S L D A E G I S G S N S F S F I E S P G K G Y A
 ACTTGGGACATCATCTAGCCGTTTCACAACTGGGGTTCAGTTAGCCCTCTCACTGGAGCAGAGGSCATTTCAGGCTCAATAGCTTTTCTTTCATTGAGAGCCCAAGGAGGATGA
 2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760

I V D E P F S E I P R Q G F L G E I R C N S E S S V L S A H E S C L R A P N L I
 CAATTGTTGATGAGCATTTCTCAGAAATCTCTCGGCAAGGGTCTTGGGGGAGATCAGGTGCAATTCAGATCTCTCAGTCTGAGTGGTCTCATGAATCATGCTTAGGCGCCCAACCTTA
 2770 2780 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880

S Y K P M I D Q L E C T T N L I D P F V V F E R G S L P Q T R N D K T F A A S K
 TCTATACAGGCCCATGATAGATCAATGAGTGGCAGCAACAATCTGATTGATCCCTTTGTGTCTTTGAGAGGGGTCTCTGCCAGAGCAAGGAATGAGCAAACTTTGCACTTCAA
 2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000

G N R G V Q A F S K G S V Q A D L T L M F D N F E V D F V G A A V S C D A A F L
 AAGGAATAGAGGTGTTCAGGCTTTCTTAAGGGCTCTGTACAAGCTGATCTAAGCTGATGTTTACAAATTTTGAAGTGGACTTTTGGGAGCAGCGTATCTTTGTGATGCCGCTTCT
 3010 3020 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120

N L T G C Y S C N A G A R V C L S I T S T G T G S L S A H N K D G S L H I V L P
 TAAATTTGACAGGTTGCTATTCTTGCATGCAAGGGGCCAGGGTCTGCTGTCTATCACATCCACAGGAAGTGGATCTCTCTGCCCACAAATAGGATGGGTCTCTGATATAGTCTTC
 3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240

S E N G T K D Q C Q I L H F I V P E V E E E F H Y S C D G D E R P L L V K G T L
 CATCAGAGATGGAAACAAAGACCAAGTGTGAGATACATACACTTCAGTGTGCTGAAGTAGAGGAGGAGTTTATGTACTCTTGTGATGGAGATGAGCGGCTCTGTTGGTGAAGGGGACCC
 3250 3260 3270 3280 3290 3300 3310 3320 3330 3340 3350 3360

I A I D P F D D R R E A G G E S T V V N P K S G S W N F F D W F S G L M S W F G
 TGATAGCCATTGATCCATTTGATGATAGGCGGGAAGCAGGGGGGGAATCAACAGTTGTAATCCAAATCTGGATCTTGGAAATTTCTTTGAGTGGTTTTCAGACTCATGAGTTGTTG
 3370 3380 3390 3400 3410 3420 3430 3440 3450 3460 3470 3480

G P L K T I L L I C L Y V A L S I G L F F L L I Y L G G T G L S K M W L A A T K
 GAGGGCTCTTAAAGCTACTCTCTTGGCTGTATGTTGATATATCAATTTGGGCTCTTTTCTCTCTTATATATCTTGGAGGAACAGGCTCTCTAAAGTGGCTTGTGCTGCTGCT
 3490 3500 3510 3520 3530 3540 3550 3560 3570 3580 3590 3600

K A S *
 AGAAGGCCCTCATAGATCAGTGGGTGAAGGAATATGTTGAAGTAAGTAGACATAAGCTAACCTAATTTATGTAAGTATTTGACAGATAGGTCAAATTTATGGAATATCCAGCTTAGAA
 3610 3620 3630 3640 3650 3660 3670 3680 3690 3700 3710 3720

ACTTATGAATAATACCTTTAGATGTAAGCTTAGTTGTAATTTGGGGTGGTGGGGTGGGAGCAGCAGCTCTCAAGTGTCTTGTGAATATCTTGTGTTGGGTAATCTCTTTTGGCAGTTA
 3730 3740 3750 3760 3770 3780 3790 3800 3810 3820 3830 3840

GCTGGGAATTAACCTAAGCTTTTGAAGTTCACCGGCTTTTGTGT
 3850 3860 3870 3880

glycoproteins are conserved between them. As shown for PT and the RVFV ZZ501 glycoproteins there are multiple hydrophobic sequences in the M12 M gene product, including a 22-28 amino acid carboxy-proximal, hydrophobic region (G1). This hydrophobic sequence is followed by a 11-amino acid-terminal sequence that contains 3 charged (basic) amino acids. The size and constitution of the carboxy-terminal region is consistent with a transmembranal and anchor function for this glycoprotein in the viral envelope. Other regions of the glycoprotein precursor contain sequences of amino acids with a predominantly hydrophobic character. Their functions are unknown.

Comparison has been made between the reported sequence of ZZ501 M RNA [and its gene product as amended by recent data from USAMRIID] and that of the M12 mutant of ZZ548, recognising that ZZ501 is not the parent of the mutant. Between the two sequences there are XX nucleotide differences and YY amino acid changes. Considering the fact that the M12 virus was derived by consecutive high level mutagenesis of another RVFV strain, these differences are small. Comparison with the sequence of the parent ZZ548 is under analysis at the present time.

Expression of the PT N and NS proteins using baculovirus expression vectors

An essentially complete DNA copy of the ambisense S RNA species of PT phlebovirus [Ihara *et al.*, 1984a] has been inserted in either orientation into *Autographa californica* nuclear polyhedrosis baculovirus (AcNPV) in lieu of the 5' coding region of the AcNPV polyhedrin gene [Overton *et al.*, 1987]. The recombinant viruses were generated using what proved to be proficient, but non-optimal expression vectors [e.g., pAcRP6, see below]. The two types of recombinant viruses were used to infect *Spodoptera frugiperda* cells and the expressed PT viral proteins characterised (Overton *et al.*, 1987). Recombinant AcNPV having the S DNA in one orientation expressed PT virus N protein in amounts estimated to represent some 50% of the infected cell extracts, whereas recombinants with the PT S DNA in the other orientation expressed the PT virus NS_S protein in significantly lower quantities. Antisera that were monospecific with respect to each of the two PT proteins virus were raised in mice using the corresponding *S. frugiperda* infected cell extracts and were employed to identify N and NS_S proteins in PT virus-infected Vero cells. These studies demonstrated a

low amount of PT NSs protein in virions and virion-derived nucleocapsids (Overton *et al.*, 1987).

The results reported previously by this laboratory have now been extended by insertion of each individual gene in a more efficient expression vector derived in our laboratory [pAcYMI, Matsuura *et al.*, 1987]. The level of expression of the PT N protein was similar to that obtained with the pAcRP6 vector, however the level of expression of the NSs protein was considerably enhanced, approaching that of the N protein. When these analyses are complete they will be prepared for publication.

Expression of PT virus glycoproteins from partial cDNA clones inserted into a vaccinia vector

Partial cDNA clones of the PT M RNA were inserted into the genome of vaccinia virus so that the PT gene products were under the control of an early vaccinia promoter. The PT sequences included in the constructs represented the entire G1 and G2 coding sequences preceded by some 24-37 upstream amino acids [depending on the constructions]. Recombinant vaccinia viruses were prepared using vaccinia plasmid pSC11 and transfection of CV-1 cells previously infected with strain IHD-J of vaccinia virus. Recombinant viruses were selected by the appropriate procedures [Smith and Moss, 1983]. In CV-1 cells the recombinants expressed glycosylated derivatives of both PT G1 and G2 proteins. In HeLa T4⁺ cells the glycoproteins were identified in the Golgi apparatus. These studies were undertaken in conjunction with Ms Y. Matsuoka and Professor R.W. Compans of the University of Alabama in Birmingham [Matsuoka, Ihara, Bishop & Compans, 1988].

C. Summary of Progress Report

The sequence of the M RNA of the M12 derivative of ZZ548 has been determined and compared to that of ZZ501. The data indicate that the RNA and its gene product are comparable for both viruses. Comparison with the sequence of the parent ZZ548 virus is in hand.

Expression of the PT N and NS_s proteins has been achieved using baculovirus expression vectors based on AcNPV. High level expression of both proteins has now been achieved. Expression of the PT M gene products, G1 and G2, in vaccinia vectors has also been achieved

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